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RESEARCH

Using Ground-Based Reflectance Measurements as Selection Criteria for Drought- and Aflatoxin-Resistant Peanut Genotypes

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ABSTRACT

Drought stress and aflatoxin contamination continue to challenge peanut (*Arachis hypogaea* L.) producers across the USA. Thus, the continued development of drought- and aflatoxin-resistant peanut cultivars is essential to maintain productivity under less than ideal growing conditions. Remote sensing of canopy reflectance is a well-established method of evaluating crop condition and shows promise as a tool for rapid selection of drought- and aflatoxin-resistant peanut genotypes. The objective of this study was to evaluate ground-based reflectance measurements to more accurately quantify differences in genotype response to drought conditions. In April 2004 and 2005 several small plots (4 m × 2 m) were established at the Gibbs Farm research facilities in Tifton, GA. Treatments consisted of five peanut genotypes encompassing a range of drought tolerance and yield characteristics. Drought conditions were simulated beginning 90 d after planting and maintained through harvest. Once drought conditions were established, a handheld radiometer was used to acquire twice-weekly reflectance measurements in the visible and near-infrared. Benchmark indices were developed based on the change in remotely sensed vegetation indices as a measure of the change in crop response between nonstressed and drought-stressed conditions. Significant treatment differences in benchmark indices were observed between drought-tolerant, moderately drought-tolerant and drought-intolerant varieties. Benchmark indices were also highly correlated with yield (r = −0.41 to −0.75, α = 0.05) in all three planting environments. However, the relationship between aflatoxin contamination and benchmark indices was less consistent, having a strong correlation with aflatoxin contamination in the second and third planting environments only (r = 0.38–0.73, α = 0.05). These indices could aid plant breeders in more accurately assessing genetic differences, which would accelerate breeding progress and the development of peanut cultivars with resistance to drought and aflatoxin contamination.

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Abbreviations: BI, benchmark indices; GNDVI, greenness normalized difference vegetation index; LAI, leaf area index; MIR, middle infrared; NDVI, normalized difference vegetation index; NIR, near infrared; NRI, nitrogen reflectance index; OSAVI, optimized soil-adjusted vegetation index; RS, remote sensing; SAVI, soil adjusted vegetation index; SRC, spectral response curve; TSAVI, transformed soil-adjusted vegetation index; TSWV, tomato spotted wilt virus; VIS, visible.

Literature is replete with field studies designed to capture plant physiological response to aflatoxin contamination (Zambettakis et al., 1981; Blankenship et al., 1985; Kisyombe et al., 1985; Mehan et al., 1986; Waliyar et al., 1994; Anderson et al., 1995). However, because aflatoxin contamination is spatially and temporally dynamic, crop response under field conditions has produced mixed results. Thus, an indirect measure of aflatoxin resistance may prove more useful. In a recent study, Holbrook et al. (2000) evaluated resistance to preharvest aflatoxin contamination in a set of peanut genotypes that had been documented as having varying levels of drought tolerance (Ruckers et al., 1995) and concluded that tolerant genotypes also had greatly reduced aflatoxin contamination. Data suggest that drought tolerance may be an effective, indirect, cost-effective selection tool for resistance to preharvest aflatoxin contamination.

However, to maximize breeding potential and reduce screening costs, field characterization for drought and aflatoxin resistance necessitates a quantitative index sufficient to differentiate among moderately resistant genotypes with potentially higher expected

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yields. Ground-based remote sensing (RS) technologies show promise as a new, unbiased, and rapid method to quantify differences in genotypic response to drought stress and aflatoxin contamination.

The amount of incoming solar energy intercepted by a plant canopy is a function of canopy structure, which is affected by disease, nutrient availability, pests, and water (Moran et al., 1994). In general, living vegetation exhibits a characteristic spectral response curve (SRC), peaking in the green and then steadily rising to maximum reflectance in the near infrared (NIR) (Hatfield and Pinter, 1993; Osborne et al., 1994; Daughtry et al., 2000). Gausman and Allen (1973) found chlorophyll concentration was the principle determinant of reflected energy from the green region of the light spectrum, while intercellular constituents, particularly water, impacted reflectance in the NIR and middle infrared (MIR) (Hatfield and Pinter, 1993).

As a result, vegetative indices designed to capture differences in plant spectra in the visible (VIS) spectrum and NIR have been developed to facilitate estimates of temporal and spatial variability of crop productivity. Three indices in common practice today are the nitrogen reflectance index (NRI) (Osborne et al., 1994), normalized difference vegetation index (NDVI) (Rouse et al., 1974), and the greenness normalized difference vegetation index (GNDVI) (Gitelson et al., 1996).

A significant body of research exists that demonstrates the utility of reflectance data for crop management. In a 2-yr study, Li et al. (2001) evaluated ground-based reflectance measurements to remotely characterize cotton (*Gossypium hirsutum* L.) response to variable water and N treatments. Data showed that the NDVI was highly correlated with biomass ($r = 0.80$, $\alpha = 0.01$) and N uptake ($r = 0.84$, $\alpha = 0.01$). Data also showed that spectral response, soil water content, N uptake and yield were affected by irrigation amount. Similar reports were made by Plant et al. (2000) showing lower NDVI values where water and N were limiting. In a recent study, Sullivan et al. (2004) successfully used plant spectral signatures to rapidly assess corn (*Zea mays* L.) yield and N status. Ground-based remotely sensed data in the 450- to 690-nm spectral region were correlated with N content as early as the V8 growth stage. However, vegetation indices, which minimize the effects of atmosphere, sun angle, and shadow improved this relationship ($r = 0.43$–0.94, $\alpha = 0.05$) throughout the vegetative and reproductive growth stages. Variability in the correlation between vegetation indices and N content over time were attributable to canopy development, water stress, and N uptake/distribution (Strachan et al., 2002).

Although literature sources are replete regarding the use of vegetation indices, attention to the use and limitations of vegetation indices is necessary. In a recent study, Ritchie and Bednardz (2005) evaluated several combinations of VIS and NIR spectra as a means to calculate the NDVI for a cotton canopy. Results indicate that the NDVI, when calculated using green or red spectra, plateaus at leaf area index (LAI) values greater than 1.5. However, the relationship between LAI and NDVI was linear when the NDVI was calculated using red edge spectra (750–850 nm). Despite this finding, earlier work by Jackson et al. (2002) demonstrated that there was utility in the NDVI for specific canopy types during the 2002 Soil Moisture Experiment in the southern Great Plains.

While much research exists to document spectral characteristics of cotton and corn, very few studies have established the relationship between spectral reflectance and pod yield, leaf area, or aflatoxin contamination in peanut. Some of the earliest work using RS technologies in peanut was established to better evaluate fungicide efficacy for leafspot (*Cercosporidium personatum*) control (Nutter et al., 1990; Aquino et al., 1992; Nutter and Littrell, 1996). Early work by Nutter et al. (1990) compared ground-based NIR reflectance measurements to visual assessments of canopy response under various fungicide treatments. A negative linear relationship between increasing NIR reflectance (800 nm) and visual assessments of disease was observed in nearly all cases, demonstrating crop vigor (low visual rating) was well correlated with increasing NIR reflectance. Moreover, Nutter et al. (1990) demonstrated that reflectance measurements explained more of the variability in leafspot and pod yield compared to visual ratings. Aquino et al. (1992) reported similar findings, showing that reflectance at 800 nm decreased with increasing disease severity and defoliation. Results from Aquino et al. (1992) showed that NIR reflectance could be used to explain 77 to 95% of the variability in green leaf area.

Because drought stress and aflatoxin contamination are a persistent challenge to peanut production, the development and release of new peanut cultivars with increased resistance to drought and aflatoxin are a necessity. While laboratory screening for aflatoxin contamination can be cost prohibitive, field characterization of drought tolerance shows promise as an indirect indicator of aflatoxin resistance. Remote sensing of canopy response is a well-documented tool to identify in-field crop stress; however, little research has been done to evaluate the utility of RS as a selection tool in a peanut breeding program. The goals of this study were to evaluate crop spectral signatures as a means to select drought-tolerant peanut genotypes and determine if drought tolerance could serve as an indirect indicator of aflatoxin resistance.

MATERIALS AND METHODS

Study Site

Five peanut genotypes were planted in Tifton, GA, on 7 May 2004 (the first planting environment [PE 1]), 21 June 2004 (the second planting environment [PE 2]), and 9 May 2005 (the third planting environment [PE 3]) in a randomized complete block design with four replications. Genotypes consisted of five

Table 1. Drought and aflatoxin characteristics for each peanut (*Arachis hypogaea* L.) genotype.

Core collection number	Plant inventory number	Aflatoxin contamination	Drought tolerance
211CC	PI 261911	Susceptible	Susceptible
375CC	PI 313123	Moderately resistant	Moderately susceptible
511CC	PI 288129	Resistant	Tolerant
522CC	PI 288099	Resistant	Susceptible
645CC	PI 461440	Susceptible	Susceptible
AT201	Check cultivar	Resistant	Tolerant

varieties having a range in expected yield, aflatoxin resistance, and drought tolerance (Table 1). Because of limited seed availability, '211CC' was replaced with '645CC' during PE 2 and PE 3. All treatments were planted in a conventional tillage system on a Tifton loamy sand (fine-loamy, siliceous, thermic, Plinthic Kandiudult). Seeds were planted in four-row plots, 2 m long, with a seeding rate of four seeds per 30 cm linear row.

Inoculum of *Aspergillus flavus* Link ex Fries (NRRL 3557) and *A. parasiticus* (NRRL 2999) was prepared and introduced to test plots 60 d after planting to ensure the presence of sufficient aflatoxin producing fungi in the peanut pod zone. *Aspergillus* inoculum was prepared using the organic matrix method (Will et al., 1994).

Drought stress was induced by covering the entire test with a mobile greenhouse (Atlas Greenhouse Systems, Alapaha, GA) on 4 Aug. 2004 (PE 1), 10 Sept. 2004 (PE 2), and 9 Aug. 2005 (PE 3). These dates correspond with approximately 90 d after planting. Mobile greenhouses remained in place for the duration of the growing season.

Plots were hand harvested on 10 Sept. 2004 (PE 1), 21 Oct. 2004 (PE 2), and 21 Oct. 2005 (PE 3). Harvested pods were dried to 7% moisture and hand sorted to remove and discard visibly damaged pods. Peanuts were shelled using a Penco peanut sheller (Peerless Engineering Company, Chual, GA) and ground in a household food processor for approximately 1 min. Aflatoxin concentration was measured on a 100-g subsample with the immunoaffinity column fluorometer method (Trucksess et al., 1991).

Ground Truth

Volumetric surface soil water content (θ_v) was collected at two depths (0–8, 8–16 cm) from the center of each plot. Volumetric soil water contents were collected coincident with each RS acquisition using a Wet Sensor Probe (Dynamax, Inc., Houston, TX). (*Note:* Use of a particular product does not indicate endorsement by the USDA Agricultural Research Service.) The Wet Sensor Probe uses a measure of the dielectric constant of the soil matrix to estimate volumetric water content (Topp et al., 1980; Whalley, 1993). The general equation can be solved to estimate volumetric water content:

$$\sqrt{\varepsilon} = a_0 + a_1(\theta_v) \quad [1]$$

where $\sqrt{\varepsilon}$ is the square root of the dielectric constant, θ_v is volumetric soil water content, a_0 is the intercept, and a_1 is the slope. Using default calibration parameters for a mineral soil, the Wet Sensor has an accuracy of ± 3 to 5% volumetric water content.

Visual ratings of crop response were also collected during each RS data acquisition as the standard measure of drought tolerance. Visual ratings were made on a scale of 1 to 5, 1 signifying a healthy canopy and 5 a severely stressed or dying canopy. Because visual ratings are subjective, a single person was selected to make ratings throughout the study.

Table 2. Specifications for the CropScan Multispectral Radiometer (1.0-m spatial resolution).

Wavelength	Band	Spectrum region†
nm		
485 ± 45	B1	Blue
560 ± 40	B2	Green
650 ± 20	B3	Red
660 ± 30	B4	Red
830 ± 70	B5	NIR
850 ± 35	B6	NIR
1240 ± 6	B7	MIR
1640 ± 8	B8	MIR
1650 ± 100	B9	MIR

†Blue, visible blue; green, visible green; red, visible red; NIR, near infrared; MIR, middle infrared.

Remote Sensing

Reflectance measurements were collected using a handheld CropScan Multispectral Radiometer (CropScan, Inc., MN). The CropScan utilizes narrowband interference filters to select discrete bands in the VIS and NIR regions of the electromagnetic spectrum. Nine bands were measured in this study within the 485- to 1650-nm range (Table 2). The CropScan is equipped with upward- and downward-looking sensors in each band and simultaneously acquires irradiance as well as radiance over the target. It is assumed that irradiance over the sensor head is equal to irradiance over the target. Radiance and irradiance were measured in millivolts, adjusted for temperature of the Cropscan, and converted to an energy term. Percent reflectance was determined using the following equation:

$$(\text{Radiance}/\text{Irradiance})100 = \%\ \text{Reflectance} \quad [2]$$

All plot data were collected as close to solar noon as possible, under clear conditions. Mobile greenhouses were removed before each RS data acquisition. Data were collected at nadir, over row middles, from a height of 2 m to approximate a 1-m^2 spatial resolution on the ground. Two measurements were collected over the center of each plot and averaged. Remotely sensed data acquisitions commenced just before inducing drought and were collected twice weekly through harvest.

Vegetation indices designed to reduce the impact of atmospheric attenuation, illumination, and bare soil contributions were used to quantify differences in crop response to induced drought. Indices included two commonly used vegetation indices: the greenness normalized difference index (GNDVI) (Gitelson et al., 1996) and the normalized difference vegetation index (NDVI) (Rouse et al., 1974). The GNDVI is calculated as

$$\text{GNDVI} = (\text{NIR}_{830\text{nm}} - \text{green}_{560\text{nm}})/(\text{NIR}_{830\text{nm}} + \text{green}_{560\text{nm}}) \quad [3]$$

where NIR corresponds to 830 ± 70 nm and green corresponds to 560 ± 40 nm, and the NDVI is calculated as

$$\text{NDVI} = (\text{NIR}_{830\text{nm}} - \text{red}_{660\text{nm}})/(\text{NIR}_{830\text{nm}} + \text{red}_{660\text{nm}}) \quad [4]$$

where red corresponds to 660 ± 30 nm. Because crop spectral response is a function of canopy structure and leaf physiology

Table 3. Soil water content (θ_v) corresponding to each remotely sensed data acquisition at 0 to 8 (shallow) and 8 to 16 (deep) cm depths. Results are listed separately for each planting environment.

Depth	PE 1			PE 2			PE 3		
	Date	θ_v		Date	θ_v		Date	θ_v	
Shallow	30 July 2004	2.55	A	9 Sept. 2004	9.55	A	9 Aug. 2005	15.32	A
	2 Aug. 2004	1.61	B	21 Sept. 2004	3.45	C	16 Aug. 2005	5.96	B
	5 Aug. 2004	1.54	B	6 Oct. 2004	2.29	D	18 Aug. 2005	5.75	B
	9 Aug. 2004	0.68	C	14 Oct. 2004	2.21	D	23 Aug. 2005	3.02	CD
	17 Aug. 2004	0.58	C	21 Oct. 2004	2.01	D	29 Aug. 2005	2.50	DE
	19 Aug. 2004	0.33	C	25 Oct. 2004	4.78	B	1 Sept. 2005	3.32	C
	24 Aug. 2004	0.43	C	1 Nov. 2004	2.45	D	6 Sept. 2005	3.32	C
							8 Sept. 2005	2.31	E
							13 Sept. 2005	1.02	F
							15 Sept. 2005	1.11	F
							20 Sept. 2005	1.31	F
	LSD	0.60		LSD	0.82		LSD	0.71	
Deep	30 July 04	2.21	A	9 Sept. 2004	11.35	A	9 Aug. 2005	15.89	A
	2 Aug. 2004	1.17	B	21 Sept. 2004	4.99	B	16 Aug. 2005	8.21	B
	5 Aug. 2004	1.11	B	6 Oct. 2004	1.99	D	18 Aug. 2005	5.75	C
	9 Aug. 2004	0.98	B	14 Oct. 2004	3.40	C	23 Aug. 2005	3.71	E
	17 Aug. 2004	0.78	BC	21 Oct. 2004	4.81	B	29 Aug. 2005	3.04	F
	19 Aug. 2004	0.89	BC	25 Oct. 2004	1.69	D	1 Sept. 2005	5.10	D
	24 Aug. 2004	0.41	C	1 Nov. 2004	2.31	D	6 Sept. 2005	4.06	E
							8 Sept. 2005	1.87	G
							13 Sept. 2005	1.25	GH
							15 Sept. 2005	1.22	H
							20 Sept. 2005	1.20	H
	LSD	0.52		LSD	0.88		LSD	0.64	

(Blackmer et al., 1994, 1996), vegetation indices were normalized to reduce variability in spectral response associated with inherent differences in pigmentation, leaf orientation, and canopy geometry between peanut varieties. To do this, benchmark indices (BI) using spectra collected before drought-induced conditions, were calculated as follows:

$$NDVI_{BI} = NDVI_{predrought} - NDVI_{drought} \quad [5]$$

$$GNDVI_{BI} = GNDVI_{predrought} - GNDVI_{drought} \quad [6]$$

where subscripts indicate the BI, predrought, and drought spectral response data. Therefore, BI represent the amount of change in NDVI or GNDVI as a function of increasing drought stress.

Data Analysis

Spectral Response Curves

Spectral response curves for each planting environment were constructed to evaluate the magnitude of change in reflectance patterns as drought conditions progressed. Because changes in spectral response were consistent throughout each measurement period, SRC were generated for four sampling periods within each planting environment: predrought, two midsampling, and before harvest.

Statistical Analyses

Data were analyzed using the Statistical Analysis System (SAS Institute, NC). Analysis of variance was used to evaluate differences in BI and soil water content for each treatment. Because differences in BI were observed over time, data were analyzed separately for each planting environment and sampling date. Duncan's least significant difference routine was used to evaluate treatment differences ($\alpha = 0.10$) and determine the magnitude of difference in BI and soil water contents between treatments.

Pearson correlation coefficients ($\alpha = 0.05$) were calculated between BI and yield or aflatoxin contamination as a method to evaluate the performance of BI in identifying high-yielding drought- and aflatoxin-resistant genotypes.

RESULTS AND DISCUSSION

Soil Water Content

Soil water content decreased steadily at both depths throughout each planting environment (Table 3). At the shallow depths, initial soil water contents were substantially lower ($\theta_{v\ shallow} = 2.6\%$) for the first planting date compared to the second and third planting dates, where initial soil water contents were 9.6 and 15.3% $\theta_{v,}$, respectively. Similar results were observed for deep soil water samples, where initial soil water contents were 9 to 13% (absolute θ_v) higher during the second and third planting dates. However, no differences in soil water content between treatments were observed. Thus, crop response to induced drought conditions was attributed to genotype resistance (or lack thereof).

Spectral Response Curves

In each planting environment, spectra acquired before inducing drought conditions exhibited a typical SRC, peaking slightly in the green (560 nm) and with a major peak in the NIR (830–850 nm) (Hatfield and Pinter, 1993; Osborne et al., 1994; Daughtry et al., 2000) (Fig. 1–3). Green spectra are closely correlated with concentrations of chlorophyll, while the peak in the NIR is more closely related to intercellular structure and water content (Hatfield and Pinter, 1993). Beyond 830 nm, spectral response declines steadily out to 1650 nm. In this region of the spectrum, soil spectra dominate the SRC. Sullivan et al. (2005) found that MIR spectra (1550–1750 nm) and thermal infrared (8200–11,220 nm) are very highly correlated with sand content, having coefficients of determination of 0.57 in Coastal Plain soils. Thus, the influence of soil background predominates this region of the light spectrum.

Initial SRC also demonstrate that plant spectral response under well-watered conditions varies as a function of genotype (Fig. 1–3). Genotypic differences in spectral response throughout the VIS, NIR, and MIR were

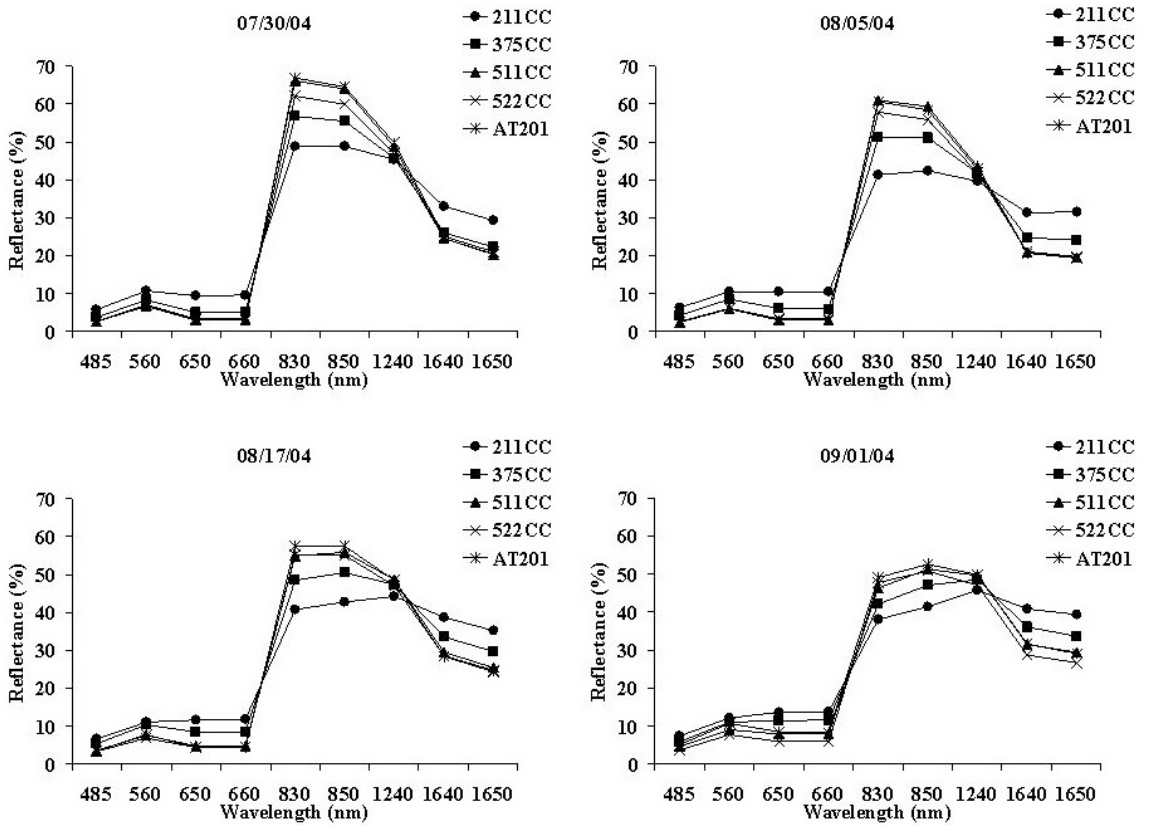

Figure 1. Spectral response curves for PE 1 are reported for predrought (30 July 2004), early drought (5 Aug. 2004), middrought (17 Aug. 2004), and late drought (1 Sept. 2004). Data represent average reflectance (%) along the *y* axis and wavelength (485–1650 nm) along the *x* axis for each treatment (211CC, 375CC, 511CC, 522CC, and AT201).

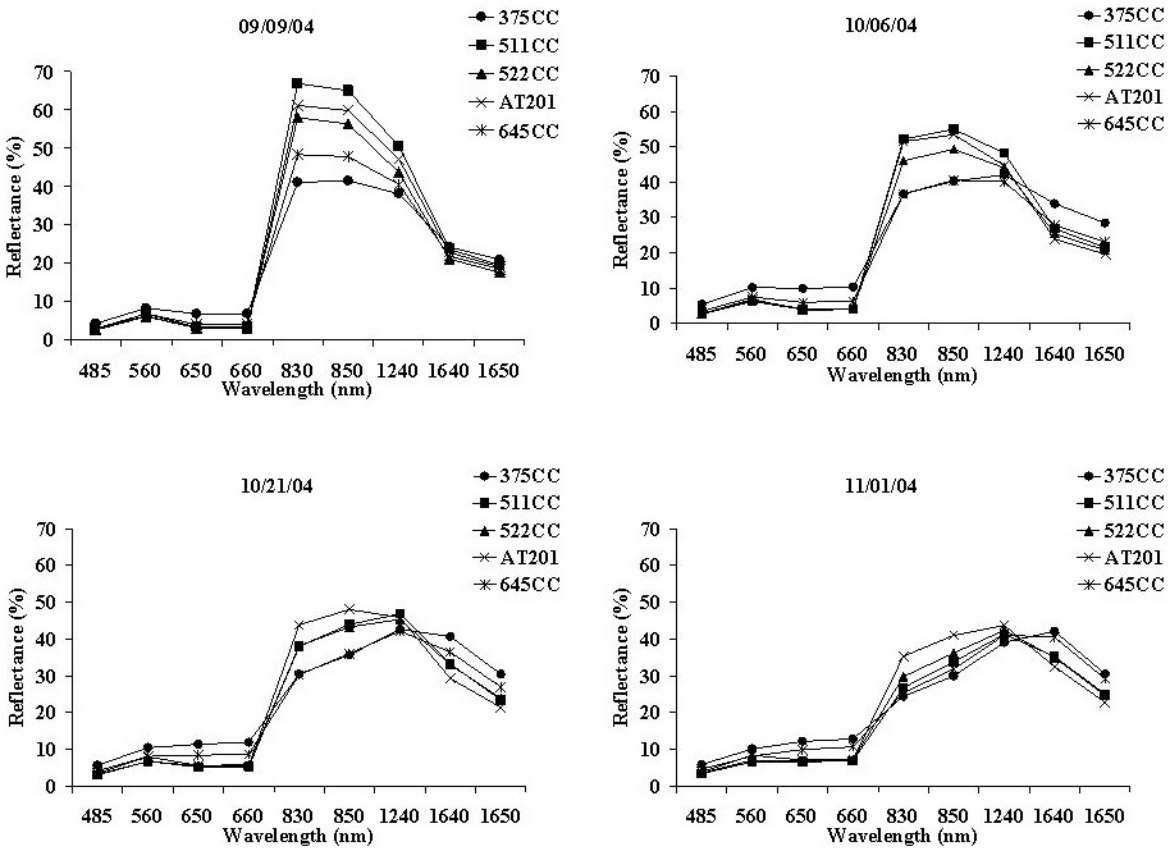

Figure 2. Spectral response curves for PE 2 are reported for predrought (9 Sept. 2004), early drought (6 Oct. 2004), middrought (21 Oct. 2004), and late drought (1 Nov. 2004). Data represent average reflectance (%) along the *y* axis and wavelength (485–1650 nm) along the *x* axis for each treatment (645CC, 375CC, 511CC, 522CC, and AT201).

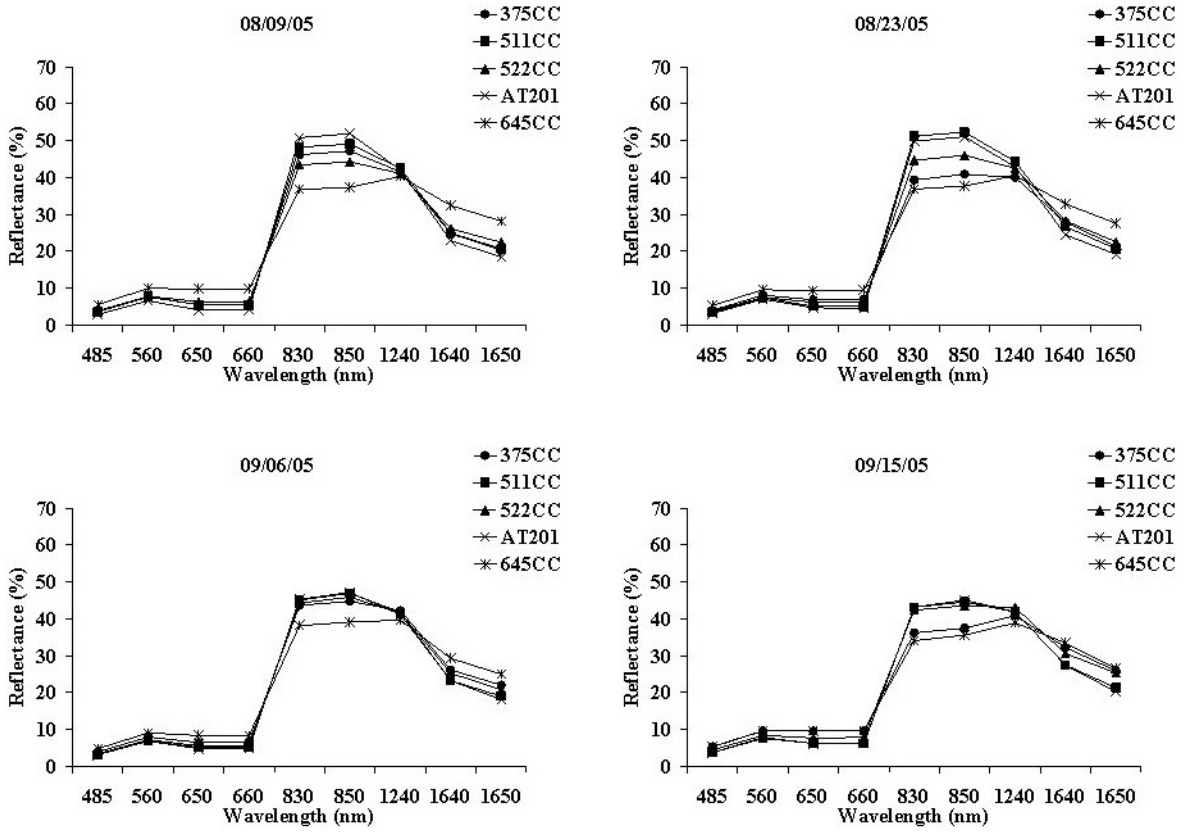

Figure 3. Spectral response curves for PE 3 are reported for predrought (9 Aug. 2005), early drought (23 Aug. 2005), middrought (6 Sept. 2005), and late drought (15 Sept. 2005). Data represent average reflectance (%) along the *y* axis and wavelength (485–1650 nm) along the *x* axis for each treatment (645CC, 375CC, 511CC, 522CC, and AT201).

attributable to inherent variability in leaf greenness, canopy development, canopy morphology, and the percentage of exposed soil within the sample area (1 m^2). Other researchers have reported similar findings, showing variability in canopy structure and morphology impact spectral response (Gallagher and Biscoe, 1978; Hatfield, 1990; Hatfield and Pinter, 1993; Daughtry et al., 2000; Li et al., 2001). In this study, initial spectral response in the green, red, and NIR during the first planting environment ranged, as a function of genotype, from 7 to 10%, 3 to 9%, and 48 to 66%, respectively. A similar range in spectral response was observed during the second and third planting environments. Thus, BI were used to normalize the dataset and better discriminate between differences in crop response to drought conditions over time.

As drought conditions intensified, the shape and magnitude of SRC changed (Fig. 1–3). During the PE 1, differences in the VIS (485–660 nm) were evident as soil water contents diminished <0.6% by volume. Red spectra increased by as much as 2 to 3%, which was likely a function of increasing soil background as the plant canopy senesced. Others have also noted the impact of soil on spectral response during periods of low canopy closure (Osborne et al., 1994; Li et al., 2001). Specifically, Li et al. (2001) showed increasing amounts of red (673–674 nm) and MIR reflectance associated with bare soil exposure during the early growth stages of a cotton crop. Changes in red spectra during PE 2 and PE 3 were evident only in the later stages of the measurement period. This is not surprising considering the higher initial soil water contents compared to PE 1 (Table 3).

Perhaps the most critical changes in plant spectra were observed in the NIR (830–850 nm). In all three planting environments, crop response to drought intensity was best expressed as a decline in NIR reflectance. Diminishing NIR spectra were observed within 3 to 12 d of inducing drought. Response was a function of initial soil water contents, with an immediate reduction of 4 to 7% in NIR reflectance during PE 1 (Fig. 1). As drought conditions persisted, NIR reflectance decreased as much as 20, 40, and 12% in PE 1, PE 2, and PE 3, respectively. In PE 3, lower initial NIR reflectance was observed due to tomato spotted wilt virus (TSWV) contamination; thus the overall reduction in NIR spectral response was considerably less compared to PE 1 and PE 2. In all cases, by the end of the measurement period the overall shape of the SRC had changed, having significantly reduced peaks in the NIR and increased reflectance in the MIR.

Benchmark Vegetation Indices

Benchmark indices were used to measure the magnitude of change in remotely sensed vegetation indices

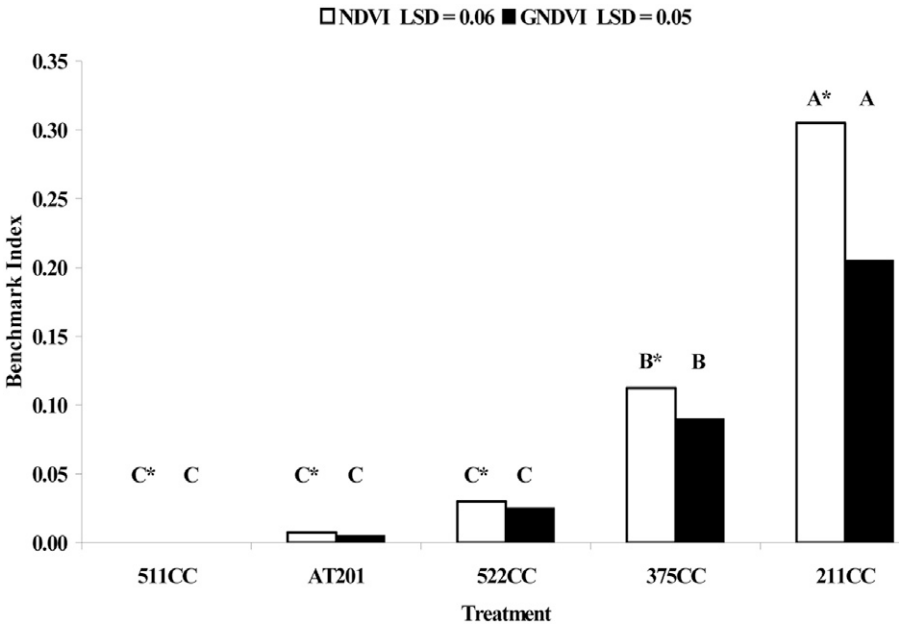

Figure 4. Data represent analysis of variance results between treatments for benchmark indices (BI) calculated during PE 1. Benchmark indices are listed along the *y* axis, and treatment along the *x* axis for a single data acquisition with a corresponding soil water content of 1.61% θ_v. Benchmark indices were calculated as follows: $NDVI_{BI}$ = $NDVI_{predrought}$ – NDVI, and $GNDVI_{BI}$ = $GNDVI_{predrought}$ – GNDVI. Means followed by the same letter are not statistically significant (α = 0.05). To differentiate between ANOVA results for the $GNDVI_{BI}$ and $NDVI_{BI}$, letters followed by an asterisk (*) denote ANOVA results for $NDVI_{BI}$.

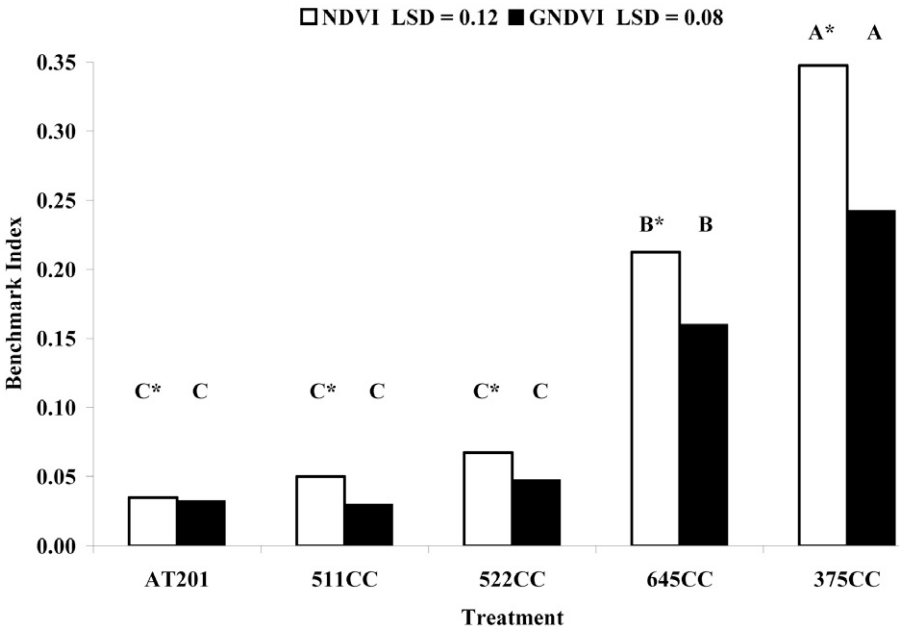

Figure 5. Data represent analysis of variance results between treatments for benchmark indices (BI) calculated during PE 2. Benchmark indices are listed along the *y* axis, and treatment along the *x* axis for a single data acquisition with a corresponding soil water content of 2.29% θ_v. Benchmark indices were calculated as follows: $NDVI_{BI}$ = $NDVI_{predrought}$ – NDVI, and $GNDVI_{BI}$ = $GNDVI_{predrought}$ – G NDVI. Means followed by the same letter are not statistically significant (α = 0.05). To differentiate between ANOVA results for the $GNDVI_{BI}$ and $NDVI_{BI}$, letters followed by an asterisk (*) denote ANOVA results for $NDVI_{BI}$.

(NDVI, GNDVI) over time. During PE 1, $NDVI_{BI}$ ranged from zero, indicating no change in canopy reflectance, to 0.43 and from zero to 0.29 for the $GNDVI_{BI}$. A similar response was observed during PE 2; however, due to TSWV infestation during PE 3, a smaller range in expected BI was observed. Due to increased levels of stress at the onset of the third measurement period, BI ranged from 0.09 to 0.35 and 0.12 to 0.25 for the $NDVI_{BI}$ and $GNDVI_{BI}$, respectively. However, cultivar AT201, which may have had increased tolerance to TSWV and was less impacted by the virus, exhibited lower initial BI ($NDVI_{BI}$ and $GNDVI_{BI}$ = 0.04) compared to the other varieties. Because vegetation indices represent a value between zero and 1, our data suggest that under sustained drought, vegetation indices can decrease by as much as 29 to 43%.

Although a range in both indices was reported for each BI, the $GNDVI_{BI}$ and $NDVI_{BI}$ ranked canopy response by genotype similarly. Separation among treatments was best during PE 1 and PE 2 (Figs. 4 and 5). During PE 1, significant differences between drought-tolerant and drought-intolerant varieties were observed within 5 d of inducing drought. This is attributable to lower initial soil water contents and consequently a more rapid response to drought compared to PE 2 and PE 3. Genotypes 'AT201', '511CC', and '522CC' were significantly more tolerant, with BI < 0.03, compared with '375CC' and 211CC. Significant differences were observed between 375CC and 211CC as well, with 375CC having moderate drought tolerance (BI = 0.09–0.11) and 211CC exhibiting the greatest degree of stress with BI = 0.21 to 0.31 (Fig. 4). Using the BI as a selection criterion for drought tolerance compared well with expected drought tolerance based on previous observations (Table 1).

During PE 2, treatment differences were also observed immediately following drought-induced conditions; however, separation was best once soil water contents reached <3% water content by volume (Table 3). Treatment differences observed within 1 wk of inducing drought (θ_v = 10%) were somewhat ambiguous, identifying only the most drought susceptible variety (375CC). It

should be noted here, that 375CC exhibited moderate drought tolerance during PE 1, the reason for increased drought stress during PE 2 is unclear. As soil water contents declined (θ_v < 3%) significant treatment differences between drought-tolerant (511CC, 522CC, and AT201), moderately drought-tolerant (645CC), and drought-susceptible (375CC) varieties were observed (Fig. 5). At this stage, BI for the drought-tolerant varieties were generally <0.07 compared to BI of 0.16 to 0.21 for the moderately drought-tolerant and 0.24 to 0.35 for drought-susceptible varieties.

Significant treatment differences were not as clearly defined during PE 3 due to the confounding effects of TSWV. This is clearly noted by higher initial BI observed (0.04–0.38) compared to PE 1 and PE 2. Crop stress exacerbated by TSWV and decreasing soil water contents likely contributed to the higher BI observed. However, as soil water content decreased (θ_v < 3%), BI successfully identified 511CC and AT201 as drought tolerant and 375CC and 645CC as drought susceptible (Fig. 6).

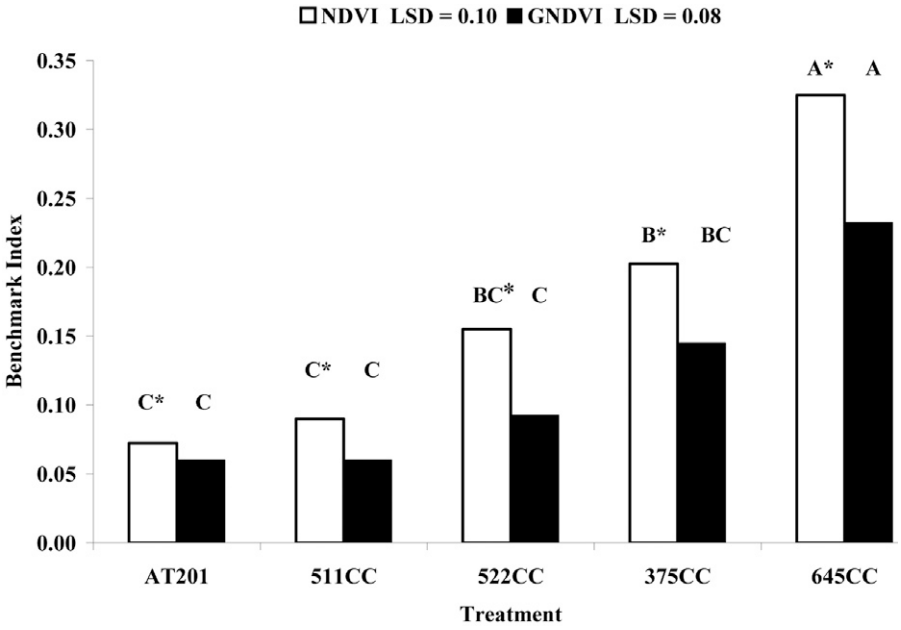

Figure 6. Data represent analysis of variance results between treatments for benchmark indices (BI) calculated during PE 3. Benchmark indices are listed along the *y* axis, and treatment along the *x* axis for a single data acquisition with a corresponding soil water content of 3.02% θ_v. Benchmark indices were calculated as follows: $NDVI_{BI}$ = $NDVI_{predrought}$ – NDVI, and $GNDVI_{BI}$ = $GNDVI_{predrought}$ – G NDVI. Means followed by the same letter are not statistically significant (α = 0.05). To differentiate between ANOVA results for the $GNDVI_{BI}$ and $NDVI_{BI}$, letters followed by an asterisk (*) denote ANOVA results for $NDVI_{BI}$.

Differentiation among drought-resistant genotypes over time is a likely function of changes in canopy geometry, senescence, and consequently, increasing contributions of soil background reflectance (Hatfield, 1990; Shanahan et al., 2001). Earlier researchers have attempted to reduce the effect of soil background reflectance, resulting in a host of additional vegetation indices including the soil adjusted vegetation index (SAVI) (Huete, 1989), optimized soil adjusted vegetation index (OSAVI) (Rondeaux et al., 1996), and the transformed soil adjusted vegetation index (TSAVI) (Baret et al., 1989). In 2001, Shanahan et al. demonstrated that the NDVI, TSAVI, and GNDVI successfully differentiated between corn hybrids and N treatments. However, correlations between yield and vegetation indices were most consistent throughout the growing season using the GNDVI. Using NDVI and TSAVI, correlations with yield were low during the initial growth stages and increased as the canopy closed. In our study, soil background effects were minimal initially and increased over time as drought conditions progressed. However, treatment differences were observed within 5 to 24 d as a function of decreasing soil water content (<3%). Moreover, treatment separation was similar using either the $NDVI_{B1}$ or $GNDVI_{B1}$. Thus, it is unlikely that soil background reflectance was a major influencing factor in determining drought tolerance in this case. Keeping this in mind, soil background effects may be an important consideration when using these data to forecast yield.

Visual Ratings

Overall, remotely sensed BI more accurately quantified drought response compared to the standard visual rating technique. Although some significant treatment differences were observed during the first planting environment, differences were inconsistent between sampling events. Within 15 d of inducing drought, visual ratings identified only 375CC and 511CC as the most drought susceptible; however, as the drought persisted, differentiation between treatments was less clear. In the second and third planting environments, visual ratings failed to identify any differences in crop response to drought.

Yield

Average yields ranged from 317 to 793 g $plot^{-1}$ across planting environments, with significantly higher yields observed during PE 2. In all three environments the most drought-tolerant genotypes generally exhibited the highest observed yields (Table 4). Among drought-tolerant genotypes, 511CC and AT201 had significantly greater yields compared to 522CC. The correlation between observed yield and BI throughout each planting environment was generally high, ranging from −0.41 to −0.75 (Table 5). Higher coefficients of variability were observed (r > −0.70) during PE 1 and PE 2. Because increasing BI are indicative of increasing stress, data demonstrate that as crop stress increases (high BI), expected yields decrease. Similar relationships between yield and vegetation indices have been reported in the literature (Blackmer et al.,

Table 4. Analysis of variance results for benchmark indices ($NDVI_{BI}$ = $NDVI_{predrought}$ – NDVI; $GNDVI_{BI}$ = $GNDVI_{predrought}$ – G NDVI), yield (g plot^{-1}), and aflatoxin (ppb). Aflatoxin data represent log-transformed values. All BI represent a single remotely sensed data acquisition, to exemplify treatment differences. This acquisition period consistently coincided with sampling times when soil water contents ranged from 1 to 3% θ_v.

Date	Variety	$NDVI_{BI}$	$GNDVI_{BI}$	Yield	Log toxin
5 Aug. 2004 (PE 1)	511CC	0.00 C	0.00 C	658.50 B	9.52 A
	522CC	0.03 C	0.03 C	360.50 C	9.24 A
	AT201	0.01 C	0.01 C	980.00 A	8.85 A
	211CC	0.31 A	0.21 A	50.00 D	6.46 B
	375CC	0.11 B	0.09 B	395.00 C	9.31 A
	LSD	0.06	0.05	125.31	1.45
6 Oct. 2004 (PE 2)	511CC	0.05 C	0.03 C	1274.50 A	7.97 A
	522CC	0.07 C	0.05 C	771.00 B	7.08 AB
	AT201	0.04 C	0.03 C	1111.00 A	6.61 ABC
	645CC	0.21 B	0.16 B	670.50 B	4.97 BC
	375CC	0.35 A	0.24 A	295.00 C	4.02 C
	LSD	0.12	0.08	295.10	2.63
23 Aug. 2005 (PE 3)	511CC	0.09 C	0.06 C	565.50 A	10.31 A
	522CC	0.16 BC	0.09 BC	120.50 B	9.57 AB
	AT201	0.07 C	0.06 C	556.00 A	9.74 A
	645CC	0.33 A	0.23 A	123.50 B	8.33 B
	375CC	0.20 B	0.15 B	221.50 B	9.11 AB
	LSD	0.10	0.08	211.35	1.40

1994; Daughtry et al., 2000; Bausch and Diker, 2001; Sullivan et al., 2004). However, only one study has demonstrated this in peanut (Nutter and Littrell, 1996), and this was associated with defoliation of the plant canopy.

During PE 1 and PE 3, the correlation between yield and $NDVI_{BI}$ generally resulted in higher correlation coefficients compared to the $GNDVI_{BI}$. This is likely due to the fact that the NDVI is less influenced by bare soil background compared to the GNDVI (Daughtry et al., 2000). Because canopy losses were greater as a function of TSWV and lower soil water contents during these measurement periods, a greater percentage of bare soil was present during each sampling event. This was particularly important as drought conditions persisted and canopy losses increased. Data from PE 1 and PE 3 exemplify this effect as a downward trending correlation between BI and yield as drought conditions worsened. However, when initial soil water contents were higher (PE 2), canopy losses and soil background effects were minimal throughout the study period. Thus, during the second planting environment the $GNDVI_{BI}$, which was more highly correlated with variability in chlorophyll content (Aparacio et al., 2000), was also more highly correlated with yield.

Aflatoxin

Because of the inherent variability in aflatoxin contamination, aflatoxin data were log transformed to normalize the dataset. Significant differences in aflatoxin contamination were observed between planting environments with PE 1 and PE 3 having significantly higher aflatoxin contamination compared to PE 2. This is not surprising and corresponds well with previous observations of TSWV contamination and low soil water contents during these periods. However, based on an analysis of variance, it was difficult to separate aflatoxin-resistant varieties using BI (Table 4).

Perhaps a better way to evaluate the potential for BI to serve as indirect indicators of aflatoxin resistance is to look at the linear relationship between BI and aflatoxin contamination. In most cases, the correlation between BI and aflatoxin contamination indicated a strong linear relationship exists between increasing aflatoxin contamination and increasing BI (r = 0.38 to 0.73) (Table 5). An exception was observed during PE 1, where correlation coefficients ranged from −0.38 to −0.47. The reasons for this are unclear and likely a function of the high degree of variability in aflatoxin contamination data observed during this planting environment. This observation is manifested in the analysis of variance results (Table 4), where only one variety (211CC) was shown to have reduced aflatoxin contamination.

CONCLUSIONS

Ground-based remotely sensed measurements were assessed as a new tool to facilitate the selection of drought- and aflatoxin-resistant peanut varieties. Field characterization of drought tolerance has typically been accomplished via a visual rating scale, and aflatoxin resistance determined in the laboratory. Because laboratory analyses can be cost prohibitive, quantitative field measures of crop response to induced drought and aflatoxin contamination may be used to streamline selection criterion and reduce the number of laboratory samples needed to evaluate aflatoxin resistance.

Benchmark indices designed to reduce the inherent variation in pigmentation and canopy structure successfully differentiated between drought-tolerant, moderately drought-tolerant, and drought-intolerant varieties under most conditions. During PE 3, BI identified only drought-tolerant and drought-intolerant varieties due to TSWV pressure that year. Performance also varied as a function of soil water content. Specifically, BI ranged from (using NDVI or GNDVI) <0.07 for drought-tolerant, to 0.07 to 0.21 for moderately drought-tolerant, and to >0.21 for drought-intolerant varieties when soil water contents reach 3% or less.

Correlations between yield and BI indicate that BI more accurately identified high-yielding, drought-tolerant varieties compared to the standard visual rating scale. Similarly, a strong linear relationship was also observed between aflatoxin contamination and BI during PE 2 and PE 3, indicating drought tolerance may also serve as an indirect indicator of aflatoxin contamination as well. However, during PE

Table 5. Correlation results relating benchmark indices ($NDVI_{BI}$ = $NDVI_{predrought}$ – NDVI; $GNDVI_{BI}$ = $GNDVI_{predrought}$ – G NDVI) to yield (g $plot^{-1}$) and aflatoxin (ppb). Data are reported for each remotely sensed data acquisition and planting environment.

Planting Environment 1				Planting Environment 2				Planting Environment 3			
Date	Index	Yield	Toxin	Date	Index	Yield	Toxin	Date	Index	Yield	Toxin
30 Jul 2004	$GNDVI_{BI}$	–	–	9 Sept 2004	$GNDVI_{BI}$	–	–	9 Aug 2005	$GNDVI_{BI}$	–	–
	$NDVI_{BI}$	–	–		$NDVI_{BI}$	–	–		$NDVI_{BI}$	–	–
2 Aug 2004	$GNDVI_{BI}$	–0.75	–0.40	21 Sept 2004	$GNDVI_{BI}$	–0.71	0.50	16 Aug 2005	$GNDVI_{BI}$	–0.52	0.70
	$NDVI_{BI}$	–0.75	–0.41		$NDVI_{BI}$	–0.69	0.48		$NDVI_{BI}$	–0.54	0.70
5 Aug 2004	$GNDVI_{BI}$	–0.70	–0.40	1 Oct 2004	$GNDVI_{BI}$	–0.73	0.52	18 Aug 2005	$GNDVI_{BI}$	–0.61	0.60
	$NDVI_{BI}$	–0.73	–0.38		$NDVI_{BI}$	–0.69	0.47		$NDVI_{BI}$	–0.63	0.60
9 Aug 2004	$GNDVI_{BI}$	–0.70	–0.40	6 Oct 2004	$GNDVI_{BI}$	–0.76	0.54	23 Aug 2005	$GNDVI_{BI}$	–0.60	0.65
	$NDVI_{BI}$	–0.73	–0.38		$NDVI_{BI}$	–0.73	0.47		$NDVI_{BI}$	–0.66	0.63
17 Aug 2004	$GNDVI_{BI}$	–0.67	–	14 Oct 2004	$GNDVI_{BI}$	–0.74	0.51	29 Aug 2005	$GNDVI_{BI}$	–0.69	0.61
	$NDVI_{BI}$	–0.74	–		$NDVI_{BI}$	–0.72	0.50		$NDVI_{BI}$	–0.71	0.59
19 Aug 2004	$GNDVI_{BI}$	–0.64	–	21 Oct 2004	$GNDVI_{BI}$	–0.75	0.51	1 Sept 2005	$GNDVI_{BI}$	–0.52	0.66
	$NDVI_{BI}$	–0.73	–		$NDVI_{BI}$	–0.75	0.44		$NDVI_{BI}$	–0.55	0.63
24 Aug 2004	$GNDVI_{BI}$	–	–	25 Oct 2004	$GNDVI_{BI}$	–0.73	0.50	6 Sept 2005	$GNDVI_{BI}$	–0.41	0.44
	$NDVI_{BI}$	–	–		$NDVI_{BI}$	–0.73	0.49		$NDVI_{BI}$	–0.46	0.42
27 Aug 2004	$GNDVI_{BI}$	–0.53	–0.39	1 Nov 2004	$GNDVI_{BI}$	–0.72	0.53	8 Sept 2005	$GNDVI_{BI}$	–0.60	0.73
	$NDVI_{BI}$	–0.63	–		$NDVI_{BI}$	–0.72	0.54		$NDVI_{BI}$	–0.65	0.71
1 Sept 2004	$GNDVI_{BI}$	–0.53	–0.47					13 Sept 2005	$GNDVI_{BI}$	–0.56	0.51
	$NDVI_{BI}$	–0.62	–0.42						$NDVI_{BI}$	–0.58	0.46
								15 Sept 2005	$GNDVI_{BI}$	–0.53	0.50
									$NDVI_{BI}$	–0.57	0.50
								20 Sept 2005	$GNDVI_{BI}$	–0.56	0.39
									$NDVI_{BI}$	–0.62	0.38

1, the observed correlation between aflatoxin contamination and BI was weak and negative, suggesting incorrectly that aflatoxin contamination was more likely to occur in less-stressed peanut canopies. Although promising, additional research is necessary to validate remotely sensed BI as an indirect indicator of aflatoxin resistance.

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